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Synergism and Antagonism in Toxicity of Mixtures of Pharmaceuticals to *Daphnia magna*

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TRINITY COLLEGE

SYNERGISM AND ANTAGONISM IN TOXICITY OF MIXTURES OF
PHARMACEUTICALS TO DAPHNIA MAGNA

BY

POOJA SHAKYA

A THESIS SUBMITTED TO
THE FACULTY OF THE ENVIRONMENTAL SCIENCE PROGRAM
IN CANDIDACY FOR THE BACCALAUREATE DEGREE
WITH HONORS IN ENVIRONMENTAL SCIENCE

ENVIRONMENTAL SCIENCE PROGRAM

HARTFORD, CONNECTICUT
6 MAY 2011

SYNERGISM AND ANTAGONISM IN TOXICITY OF MIXTURES OF
PHARMACEUTICALS TO DAPHNIA MAGNA

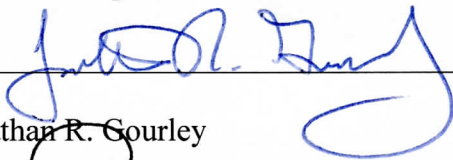
BY
POOJA SHAKYA

Honors Thesis Committee

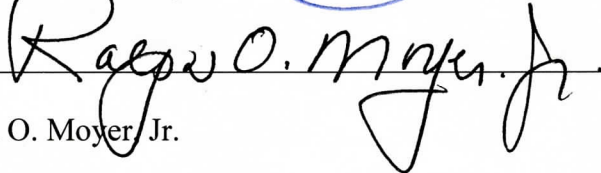
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Jonathan R. Gourley

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Ralph O. Moyer, Jr.

Date: 5 May 2011

ACKNOWLEDGEMENTS

I would firstly like to express my sincerest gratitude to Dr. Alison J. Draper. I identify her as my role model and I cannot imagine such a rich Trinity experience without her. I initially met her as my first-year advisor for the Interdisciplinary Science Program (ISP) but over the past few years Alison has stepped up to the role of a thesis advisor, mentor and a close friend. She has been a relentless source of support throughout my college career and has always shown a keen interest in helping me in any way possible. Alison's work ethics are exemplary and her confidence in me has helped me strive for my personal best. I feel very fortunate to have met her and I fully owe my passion for the process of discovery and achievements at Trinity to her.

I am very thankful to Dr. Jonathan R. Gourley for also being part of my thesis committee. I have enjoyed taking various classes with him and it has definitely helped me broaden my understanding of the environment. I also greatly appreciate Dr. Ralph O. Moyer, Jr., for being a part of my thesis committee.

I would also like to thank all my lab partners who have supported me in this project at various stages: Rachel Riendeau, Richard Kim, Rachel Lynch and Airelle A. James. I particularly appreciate the time Rachel and Richard have spent helping me over the semester and the summer. Their support, patience and great company has been invaluable to me. I am grateful for Kathy Mallinson and Michael Donagher for their prompt and constant help and support throughout the past three years.

I would also like to thank my family. Despite being so far away from home, their love and support have always been close to me throughout my college years.

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ABSTRACT

Pharmaceuticals escape wastewater treatment plants and contaminate aquatic environments; there is increasing concern about the exposure of aquatic organisms and the combined toxicity of this complex mixture of chemicals. Four human pharmaceuticals were chosen for this study: all are water-soluble and thus, complications of solvent effects are eliminated, and all are commonly used in the U.S. and have been detected in the aquatic environment. A 48-hour motility assay of <24 hour-old *Daphnia magna* neonates was used to examine the effects of a mixture of commonly-used pharmaceuticals. Due to the lack of aquatic toxicity data, LC₅₀ (lethal concentration at the 50th percentile) and NOAEL (no observed adverse effect level) concentrations of propranolol, metoprolol, terbutaline, and metformin were estimated.

The first part of this study investigates the acute toxicity of these four drugs – two beta blockers, a bronchodilator and an antihyperglycemic drug, respectively – using high concentration binary mixtures according to methods well-established in the literature. These methods are aimed at establishing the nature of the interactions, determining whether the drugs are synergistic (higher toxicity) or antagonistic (lower toxicity) when given in combination. The interaction of metformin and metoprolol was shown to be antagonistic.

The second part of this study examines mixture toxicity again, but at low, environmentally-relevant concentrations in complex mixtures. Synergism and antagonism were observed in these mixtures. In contrast to the results described for high-concentration experiments metformin and metoprolol were synergistic. Additionally, metformin and metoprolol together (but not separately) were synergistic

with propranolol. Terbutaline and propranolol were also synergistic, but the addition of either metformin or metoprolol to the mixture antagonized the combined toxicity of the terbutaline/propranolol mix. None of these relationships would be predicted by the drugs' mechanism of action in humans.

These results suggest that mixture toxicity evaluated at high (LC_{50}) concentrations may not predict the interactions observed at low, environmentally-relevant (NOAEL) concentrations. This calls into question the standard methods for evaluating mixture toxicity. Additionally, frequent use of pharmaceuticals by consumers coupled with imperfect methods of wastewater treatment will likely increase pharmaceutical residue in the aquatic environment. Future experiments will be aimed at determining the mechanism of drug interactions observed in this study. Furthermore, it should also consider the toxicological impact of degradation products of pharmaceutical mixtures on the overall aquatic ecosystem and human health.

INTRODUCTION

Pharmaceuticals and their metabolites enter the aquatic environment from leaching, excretion of human waste and through wastewater systems (Kim *et al.*, 2007; Dussault *et al.*, 2008; Nikolaou *et al.*, 2007). These substances and their metabolites have been detected in many countries in surface water, ground water, drinking water, seawater, discharge from sewage treatment plants (STP), wastewater treatment plants (WWTP), and soil sediments. Some of the drugs resist degradation and are found in the environment in their parent, active form (Fent *et al.*, 2006; Hernando *et al.*, 2006; Kim *et al.*, 2007). Unaltered or metabolized pharmaceuticals are excreted from the patient through urine and feces and end up in the sewage system. However, wastewater treatment plants fail to completely degrade all pharmaceuticals, leaving the water contaminated with some pharmaceutical drugs (Langford, 2009, Dokianakis, 2004).

With improved medical treatment and rising human population the number of prescriptions dispensed per year is also steadily increasing (Huggett *et al.*, 2003A; Huggett *et al.*, 2003B; Koplin *et al.*, 2002). This in turn increases the amount of pharmaceuticals that ends up in the environment and is an emerging concern. In 2008, the total number of drugs ordered or prescribed in the U.S. was 2.3 billion (FASTSTATS, 2011). Even if some pharmaceuticals have relatively short environmental half-lives they are assumed to be “pseudopersistents” due to their polar and non-volatile nature which prevents them from escaping the aquatic realm (Hernando *et al.*, 2006). Additionally, the constant input of pharmaceuticals may also induce toxic effects even in trace amounts due to high biological activity at the site of

action. Studies have shown that some pharmaceuticals which are specifically designed for human target receptors have resulted in sublethal toxicity (cancer and reproductive damage) in organisms with genetic similarities due to potent interaction (Koplin *et al.*, 2002).

The environmental concentrations of these drugs are usually detected at trace levels (ng/L or ppt); recent published literature supports this estimate (Kolpin *et al.*, 2002; Kolpin *et al.*, 2004; Stackelberg *et al.*, 2007; Pedrouzo *et al.*, 2008; Gómez *et al.*, 2006; Gibs *et al.*, 2007; Joakim Larsson *et al.*, 2007; Kosonen *et al.*, 2009). Although low, these concentrations may be significant enough to induce toxic and synergistic effects on aquatic species due to the drugs' continual influx and persistence in the environment (Hernando *et al.*, 2006; Isidori *et al.*, 2009; Kim *et al.*, 2007). Additionally, organisms are exposed to a mixture of chemicals in the environment. This may result in different combined effects: simple addition of a toxic effect (noninteractive or additive action), an effect which is less than the sum of the separate constituents (antagonism) and toxic effects which may exceed the total additive effects of the separate constituents (synergism) (Otitoloju, 2002).

Published toxicity data exist for some of the drugs tested; in general, LC₅₀ values are quite high but are also highly variable depending on experimental conditions (Kim *et al.*, 2007; NOAA, 2006; Park, 2005). However, even the comparatively low environmental concentrations may be significant enough to induce toxic and synergistic effects on non-target species due the continual influx and persistence in the environment (Hernando *et al.*, 2006; Isidori *et al.*, 2008; Kim *et al.*, 2009). Frequent prescriptions of drugs account for this constant influx. The full extent

of the aquatic exposure to these pharmaceuticals is still unknown. LC₅₀ values have been published for the pharmaceuticals in this study: propranolol and metoprolol have LC₅₀ values for *Daphnia magna* of 1.6 mg L⁻¹ (Dzialowski, 2006) and 64 mg L⁻¹ (Huggett, 2002) respectively.

Types of Common Pharmaceuticals

Human and veterinary drugs are continuously released into the environment from leaching/excretion of human waste, direct disposal, sewage treatment plants (STPs), manufacturing processes, animal excreta, and surface runoff (Hernando *et al.*, 2006; Kim *et al.*, 2009; Dussault *et al.*, 2007).. Many of these drugs resist degradation and remain in the environment at trace levels. However, pharmaceuticals constantly enter and persist in the environment due to the high use of drugs prescribed in the United States.

Studied Pharmaceuticals

This study focuses on four commonly prescribed, water-soluble pharmaceuticals used in the United States: two beta blockers, a bronchodilator and an anihyperglycemic drug (shown in Table 1). Beta blockers such as propranolol, metoprolol, and nadolol have been detected in European effluent discharges (Ternes, 1998). More recently the wastewater effluent samples from Texas, New York, and Mississippi detected β -adrenergic receptor antagonists at levels as high as 1.9 $\mu\text{g L}^{-1}$ (Huggett *et al.*, 2003B). The site of action for beta blockers is on β -adrenergic

receptors, a class of receptors vital for the normal function of the sympathetic branch of the vertebrate autonomic nervous system (Huggett et al. 2002).

Metoprolol (structure shown in Figure 1) is a β_1 selective andrenoceptor blocking agent which is widely used to treat high blood pressure or hypertension. It ranks as the #9 most commonly dispensed drug in the United States (Rxlist, 2011). Approximately 2% of the metoprolol entering the wastewater treatment stems from hospital effluent (Langford *et al.*, 2009). The amount of metoprolol present in sludge ranges from 0.026 $\mu\text{g/L}$ to 0.063 $\mu\text{g/L}$ with a mean of 0.039 $\mu\text{g/L}$ (Radjenović *et al.*, 2009). According to a study conducted by Huggett *et al.* (2003B), metoprolol was detected in wastewater treatment plants with the highest U.S. concentration effluent reported at 1.9 $\mu\text{g L}^{-1}$.

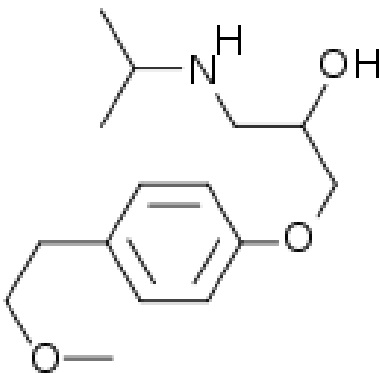


Figure 1: Example of beta blocker - Metoprolol

Propranolol (structure shown in Figure 2) is a β adrenergic receptor blocking agent which is also used to treat hypertension, angina, post-traumatic stress, and reduce the severity and frequency of migraine headaches. More than 50 million Americans are diagnosed with hypertension and more than 4 million suffer from angina (Huggett *et al.*, 2002). Around 11% of the propranolol entering the wastewater treatment comes from hospital effluent; this may cause minor inhibition on the performance of nitrite oxidizing bacteria (Langford *et al.*, 2009; Dokianakis *et al.*, 2004). The propranolol level found in sludge ranged from 0.072 $\mu\text{g/L}$ to 0.54 $\mu\text{g/L}$ with a mean of 0.347 $\mu\text{g/L}$ (Radjenović *et al.*, 2009). Furthermore, propranolol was detected in wastewater treatment plants with the highest U.S. concentration effluent reported at 1.9 $\mu\text{g L}^{-1}$ (Huggett *et al.*, 2003B).

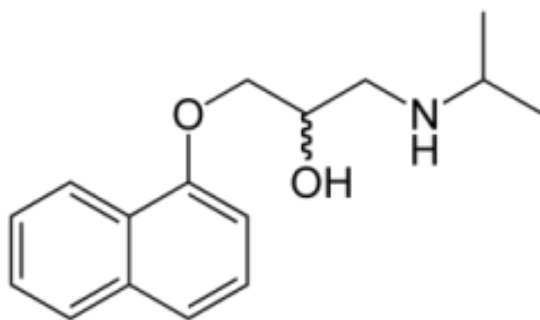


Figure 2: Example of beta blocker - Propranolol

Terbutaline (structure shown in Figure 3) is a β -adrenergic agonist bronchodilator which is used to treat asthma, chronic bronchitis, emphysema and other respiratory diseases. A study conducted by Låven *et al.* (2009) detected terbutaline in influent and effluent wastewater at a concentration of around < 0.21

$\mu\text{g/L}$ and $<0.085 \mu\text{g/L}$ respectively. Additionally, the study also showed that the concentration levels were reduced to $<0.12 \mu\text{g/L}$ in ozone treated effluent and to $<0.080 \mu\text{g/L}$ in wastewater passed through MBR (membrane bioreactor).

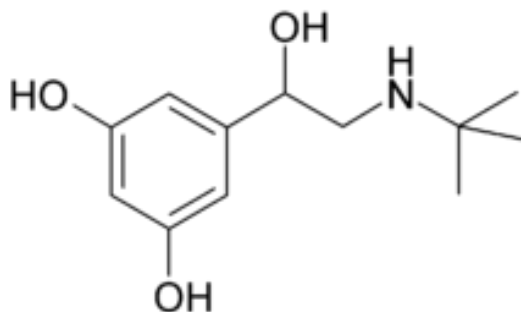


Figure 3: Example of bronchodilator - Terbutaline

Metformin (structure shown in Figure 4) is an antihyperglycemic drug which has been used to treat non-insulin-dependent (type 2) diabetes for more than 40 years (Salpeter *et al.*, 2003). Metformin was detected in U.S. stream samples with a median of $0.11 \mu\text{g/L}$ and a maximum of $0.15 \mu\text{g/L}$ (Koplin *et al.*, 2002). In the United States, metformin ranks as the 17 most commonly dispensed pharmaceutical (Rxlist, 2011).

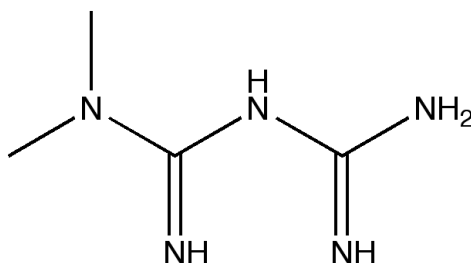


Figure 4: Example of antihyperglycemic drug – Metformin

Table 1. Pharmaceuticals tested in the current experiment.

Generic Name	Brand Name	Use	Mechanism of Action	Extent of Use¹
Metformin	FORTAMET® GLUMETZA®	Diabetic drug	Antihyperglycemic drug	# 17 by prescriptions dispensed
Metoprolol	LOPRESSOR®	Treatment of cardiovascular disease, hypertension	β1-sel blocker	# 9 by prescriptions dispensed
Propranolol	INDERAL®	Treatment of hypertension	Non-sel β-blocker	
Terbutaline	TERBUTALINE SULFATE	Bronchodilator	β 2-agonist	

¹Data obtained from Rxlist, 2011

Confounding Factors

Organisms and ecosystems are exposed to a mixture of chemicals in the environment which may result in different combined toxic effects: simple addition, synergism or antagonism. However, the complication of mixture toxicity of pharmaceuticals in the environment is still not fully understood (Otitoloju, 2002). Additionally, pharmaceuticals may reach the aquatic environment by bypassing the STP effluent, thus directly causing bioaccumulation for organisms downstream (Dokianakis, 2004). However, there is still a lack of toxicity information on the interaction of commonly used pharmaceuticals in the aquatic environment (Koplin *et al*, 2002).

Wastewater Treatment Processes

Wastewater treatment speeds up the natural process of water purification and is one of the most common forms of pollution control in the United States. The two main stages in wastewater treatment are primary and secondary treatment. Aerobic bacteria can decompose naturally occurring organic matter in the environment. However, the biological oxygen demand needed to decompose the waste produced by humans would be too high for naturally occurring bacteria without the help of wastewater treatment plants.

The primary wastewater treatment (as shown in Figure 5) starts with a bar screening process which removes twigs, rags and other large debris. Next it is passed ‘downstream’ through a circular grit chamber where the cinders, sand, and small stones are spun out. The suspended solids in the wastewater are then allowed to settle to the bottom of the sedimentation tanks and the resulting sludge is removed (US EPA, 1998; MDC, 2011).

Biological treatment is used in secondary wastewater treatment (as shown in Figure 6) because it is effective on bacteria, contaminants, fungi and protozoa. Around 85% of the organic matter in sewage is removed by using the bacteria present in the sludge (US EPA, 1998).

Primary Treatment

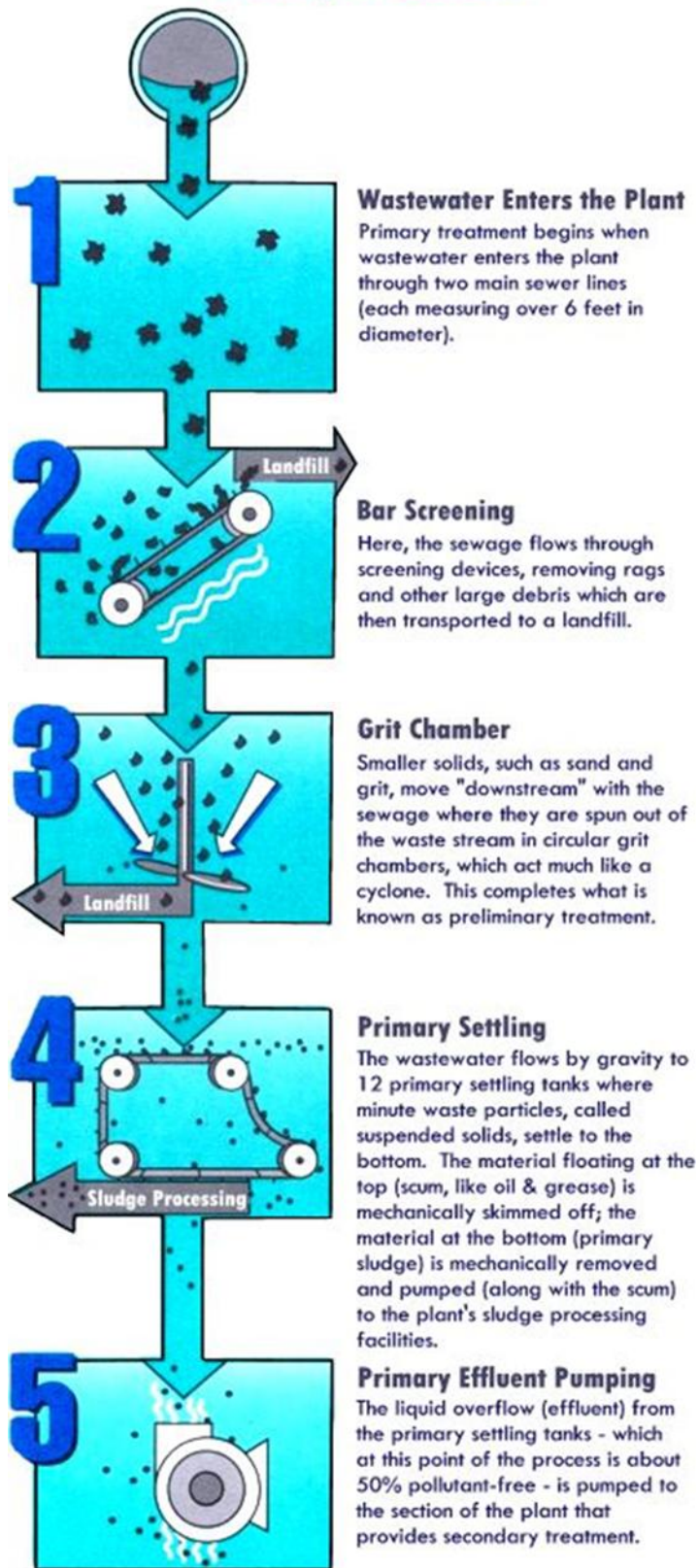


Figure 5: Primary wastewater treatment (MDC, 2011)

Secondary Treatment

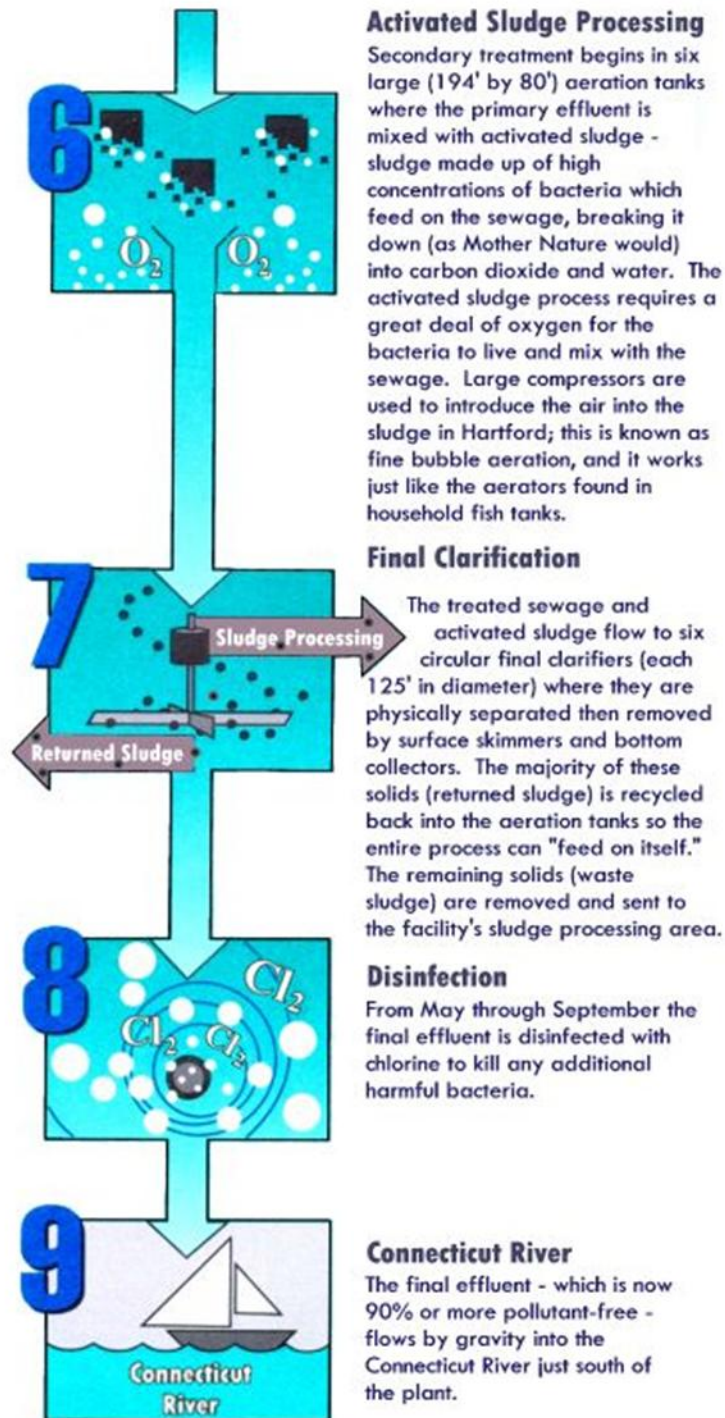


Figure 6: Secondary wastewater treatment (MDC, 2011)

Testing toxicities of mixtures

The standard method for evaluating mixture toxicity measures the toxicity of binary mixtures at high concentrations (around their LC₅₀s). However, this does not represent the ‘real’ environment. Thus, pharmaceuticals are also tested in low concentration, binary and more complex mixtures.

Analyses of ecotoxicity data

In this research study, the data obtained from the tests were analyzed with the help of a TU (Toxic Unit) model developed by Brown (1968) and Sprague (1970). This provides a graphical representation of the results. The number of toxic units associated with each of the constituents of the mixture was calculated as shown below:

$$TU_A = \frac{\% \text{ of A in mixture} \times EC50 \text{ of mixture}}{EC50 \text{ of A alone}}$$

Where TU_A: Toxic Unit of substance A in the mixture.

If the sum of the TU of all the constituents in the mixture is equal to 1 then the interactions are additive. If the sum of constituents is less than one the interactions are antagonistic and if the sum is greater than 1 the interaction is synergistic.

Isobologram representation

Sprague (1970) described a method by which the interactions of the 2-substance mixtures 'A' and 'B' could be represented in two dimensions. The curve is a straight line as shown in Figure 7(a) if the effect is additive. However as shown in Figure 7(b), if the effect is synergistic, the isobole of the AB mixture is located below the additivity isobole (lower left); whereas, if the effect is antagonistic, the isobole of the mixture is located above the isobole of additivity (Otitoloju, 2002; Warne, 2003).

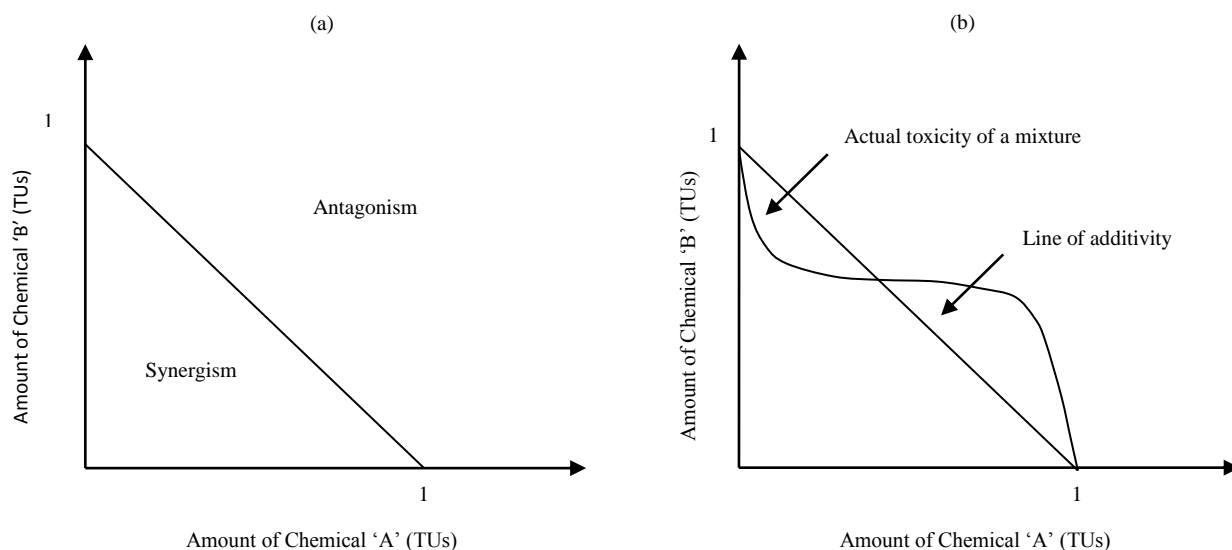


Figure 7: Example of isoboles (a) showing additivity and the domains of antagonism and synergism, (b) highlighting a variation of interactions between two substances as a function of their ratio (Warne, 2003).

In the current study, five different mixture ratios of each binary pair were prepared (20:80, 40:60, 50:50, 60:40, and 80:20). Each of these mixture ratios was tested in triplicate using six different concentrations around the mixture LC_{50} according to the method outlined by Panouillères *et al.* (2007). For the data analysis of these mixture experiments, a two-step process was used: the concentration-response relationships were determined and an isobole analysis was used to assess the interaction of the two drugs in the mixture.

RESEARCH OBJECTIVE

This research study compares the results from testing mixtures in two ways:

- 1) High concentration, binary mixtures
- 2) Low concentration, binary and more complex mixtures

High concentration, binary mixtures

The first part of the study analyzes the effects of commonly used pharmaceuticals in binary mixtures on *Daphnia magna*. First, the LC₅₀ will be determined for each drug on *Daphnia magna*. Subsequently, mixtures of pharmaceuticals will be used to assess possible synergism and antagonism using methods well-established in the literature.

Low Concentration, binary and more complex mixtures

The second part of the study seeks to better understand mixture toxicity at environmentally relevant concentrations. It is possible that interactions at low environmentally relevant concentrations will be different than those at high concentrations. This would call into question the standard methods used to assess mixture toxicity. However, there is limited information available on the toxicological effects of pharmaceuticals in the environment and it is vital to continue research to understand these interactions in greater depth.

MATERIALS AND METHODS

Stock solutions of 100g/L were prepared in hard water and stored at 4 ° C until used.

Lower concentrations were prepared by serial dilution.

- β 1 selective andrenoceptor blocking agent: Metoprolol (Sigma-Aldrich)
- β -adrenergic receptor blocking agent: Propranolol (Sigma-Aldrich)
- β -adrenergic agonist bronchodilator: Terbutaline (Sigma-Aldrich)
- Antihyperglycemic drug: Metformin (Sigma-Aldrich)

Daphnia magna

Daphnia magna were obtained from Aquatic Research Organisms (Hampton, NH). Acute toxicity tests were performed in hard water according to Standard Methods (1998). Ten neonates (<24 hr old) were placed in test tubes with 10 mL of drug solution prepared in hard water. *Daphnia* were incubated at room temperature and the motile *daphnia* were counted after 48 hrs. Each of the tests was performed in triplicate.

Determination of LC₅₀ values

Incubations were performed as described above, using 5 concentrations around the LC₅₀ value. For *Daphnia Magna*, 48-hr LC₅₀ values for the 24-hr old larvae were determined by probit analysis.

Determination of NOAEL (No Observed Adverse Effect Level)

For each drug that was used in this study the NOAEL (the highest concentration tested that causes no toxicity) was determined.

Preparation of Hard Water

Hard water was prepared according to the Standard Methods (Eaton, 1998). To prepare 4 L of hard water using DDI (distilled deionized water), the following amounts of salts (as shown in Table 2) were used:

Table 2. Amount of salt (by weight) needed to make 4 L of hard water

<u>Salt</u>	<u>Formula</u>	<u>4L</u>
Sodium bicarbonate	NaHCO_3	0.768 g
Calcium sulfate dehydrate	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.480 g
Magnesium sulfate heptahydrate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.737 g
Potassium chloride	KCl	0.032 g

The salts were added to distilled deionized water in a glass Erlenmeyer flask with a stir bar and then placed on a magnetic stir plate for at least 48 hours before experiments. Water was aerated by bubbling air through in order to equilibrate the carbonate buffer.

RESULTS AND DISCUSSION

This study exposed the test organism, *Daphnia magna*, to varying concentrations of commonly used pharmaceuticals to assess the toxicity of pharmaceuticals in the environment. The pharmaceuticals were tested in high concentration binary mixtures and at low concentration for binary and more complex mixtures.

LC₅₀ Value Determination

The LC₅₀ value was experimentally determined for each drug due to the lack of information available on the aquatic toxicity of these pharmaceuticals to *Daphnia magna* and to determine concentrations needed for mixture experiments. The LC₅₀ values determined for metformin, metoprolol and propranolol were 87.5 ppm, 175 ppm and 3.9 ppm respectively. These LC₅₀ concentrations were used in subsequent high-concentration mixture toxicity experiments.

High-concentration Mixture Toxicity

Three binary mixtures BC, BD and CD were tested, where B = Metformin, C = Metoprolol and D = propranolol. The isobole parameters for the binary mixtures tested with *Daphnia magna* are shown below.

If Figure 8 is compared to Figure 7 (b) the results indicate that the interaction between high concentration binary mixture of metformin and metoprolol is antagonistic (it lies above the line of additivity).

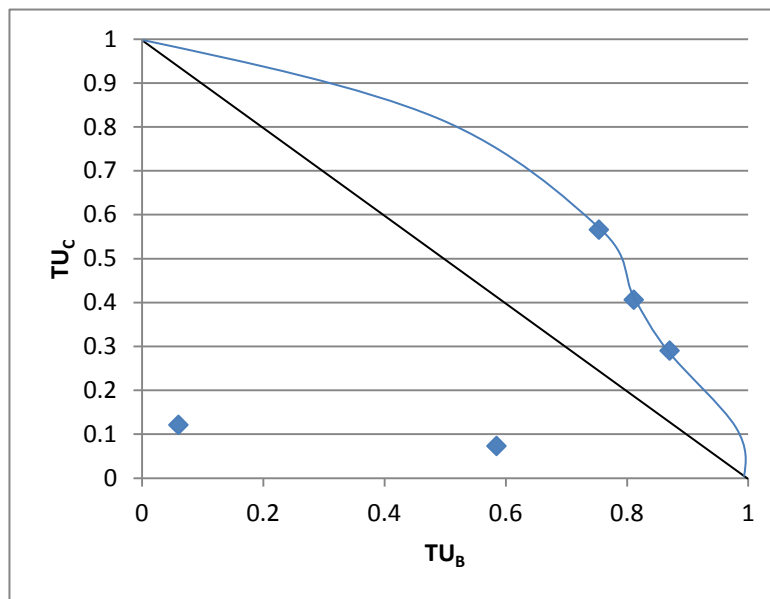


Figure 8: Isobolic representation of estimated dose combination for binary mixture of B = Metformin, C = Metoprolol, TU = Toxic Unit, tested with *Daphnia magna*.

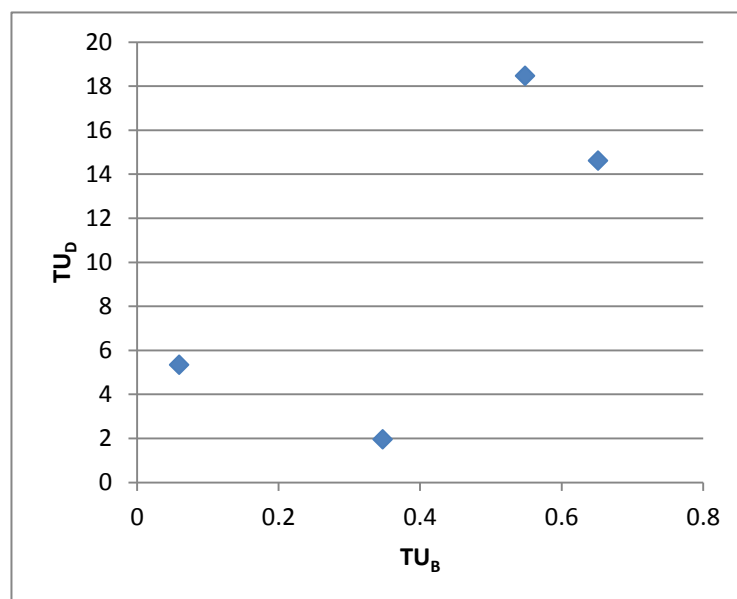


Figure 9: Isobolic representation of estimated dose combination for binary mixture of B = Metformin, D = Propranolol, TU = Toxic Unit, tested with *Daphnia magna*.

Both Figure 9 and Figure 10 show the isobolic representation for binary mixtures of BD (metformin, propranolol) and CD (metoprolol, propranolol). A line of additivity is not observed in these two figures. A possible reason for this could be that propranolol is very potent and its presence in the mixture overshadows the TU (Toxic Unit) for the other substance. Future work with terbutaline, which has an LC_{50} around the same order of magnitude as metoprolol and metformin will be helpful in elucidating additional interactions and addressing this hypothesis.

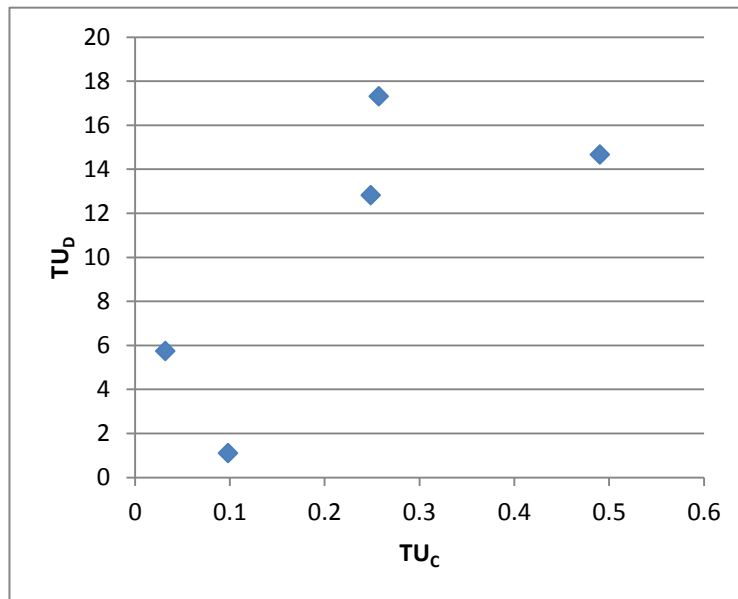


Figure 10: Isobolic representation of estimated dose combination for binary mixture of C = Metoprolol, D = Propranolol, TU = Toxic Unit, tested with *Daphnia magna*.

NOAEL Tests

The NOAEL tests were performed first on all four pharmaceuticals to determine the concentration at which the average percentage survival for *Daphnia magna* was around 100%. The results of the test determined the NOAEL levels for each drug as follows: terbutaline (100 ppm), metformin (50 ppm), metoprolol (50 ppm) and propranolol (1 ppm).

Low-concentration Mixtures

Daphnia neonates were incubated for 48 hours at room temperature with the simple and complex mixtures and survival was recorded to assess toxicity. As shown in Figure 11, almost all of the neonates survived at the NOAEL concentrations with around 100% survival. However, when metformin and metoprolol were added to terbutaline, a synergistic effect was observed and the average percentage of survival decreased to around 56%. Furthermore, adding propranolol to this mixture lowered the survival rate of *Daphnia magna* even more to around 25%. A similar synergistic effect was seen when metoprolol and propranolol were added to metformin where the average survival percentage decreased to around 38%.

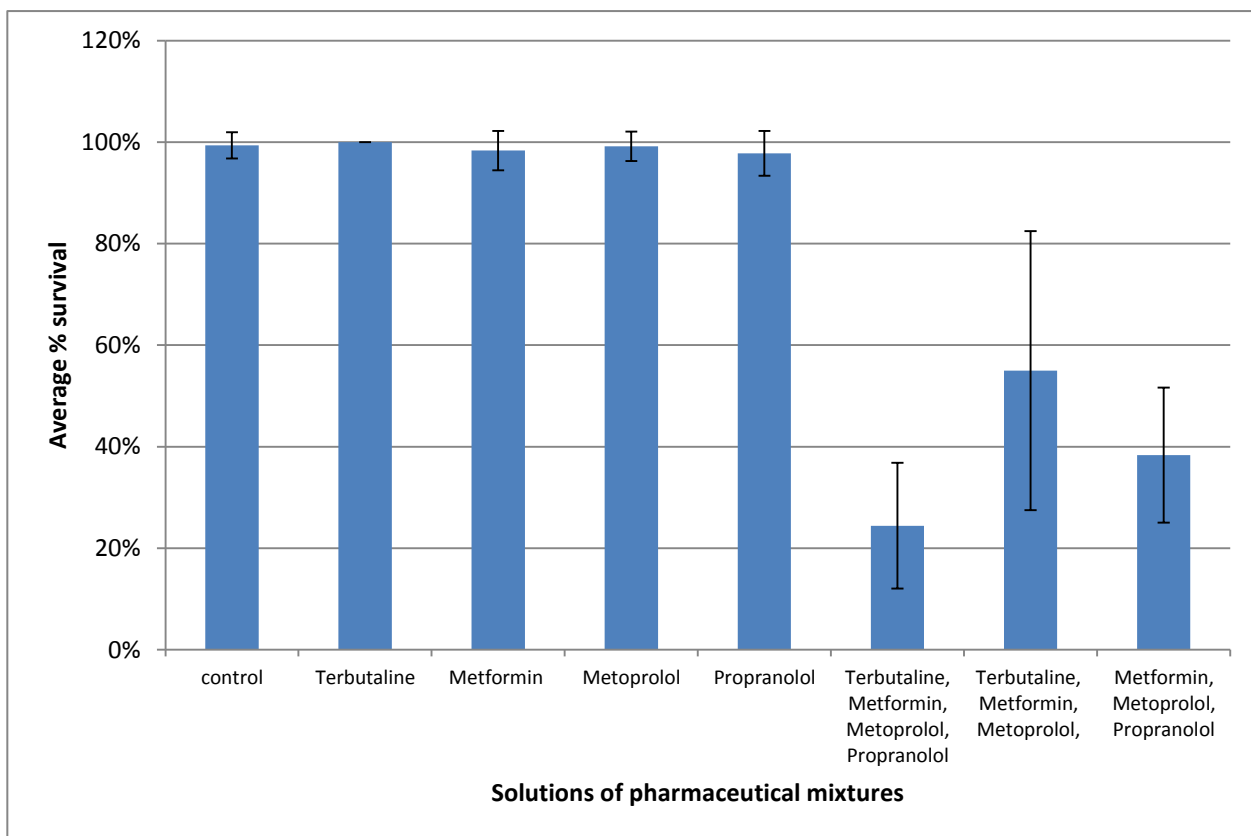


Figure 11: Synergistic effect of Terbutaline (100 ppm), Metformin (50 ppm), Metoprolol(50 ppm) and Propranolol(1 ppm) mixtures on survival of 24 hour neonate *Daphnia magna* in a 48 hour exposure.

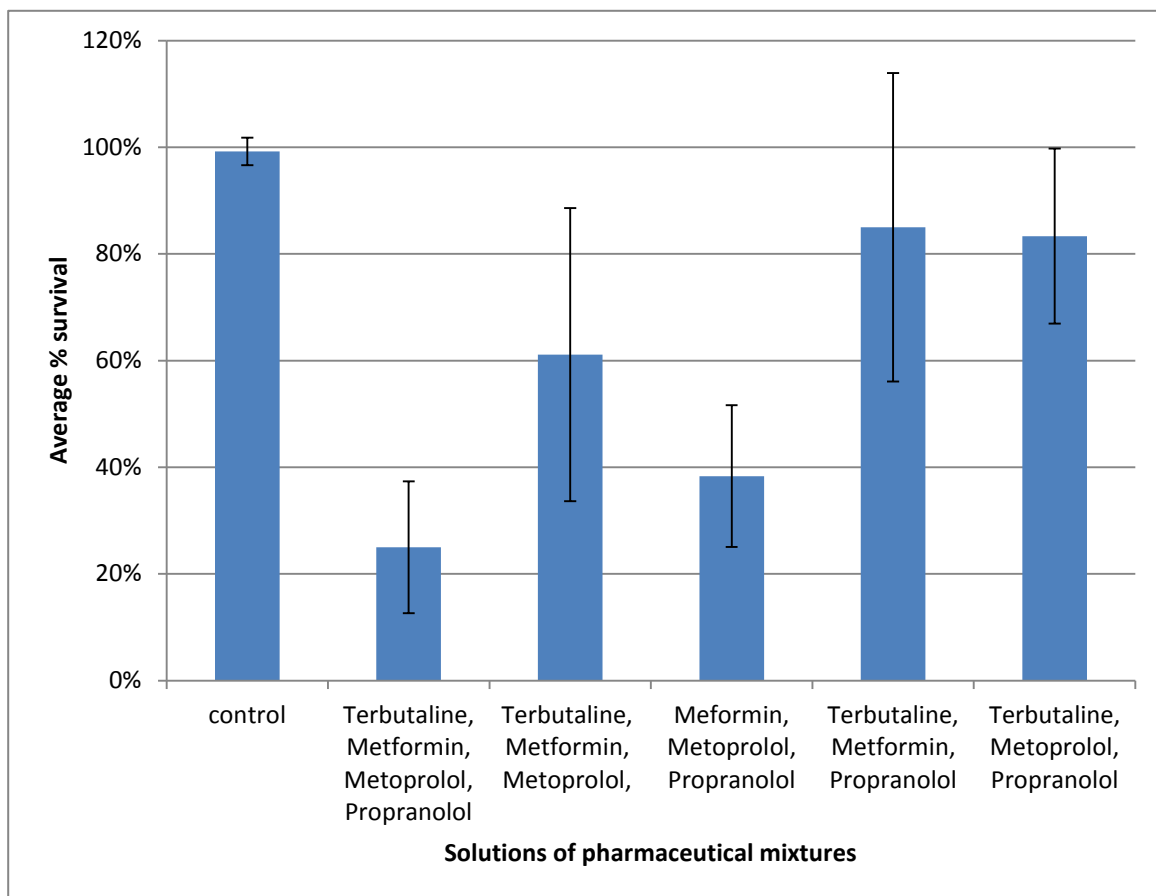


Figure 12: Synergistic effect of Terbutaline (100 ppm), Metformin (50 ppm), Metoprolol (50 ppm) and Propranolol (1 ppm) mixtures on survival of 24 hour neonate *Daphnia magna* in a 48 hour exposure.

Mixtures where metformin and metoprolol occurred together (but not individually) with propranolol demonstrated synergistic effects (see Figure 12 and Figure 13). Terbutaline and propranolol were also synergistic; however, the addition of either metformin or metoprolol to the mixture antagonized the combined toxicity (see Figure 12 and Figure 14). In the mixture of all four drugs, the synergistic effect observed was of the highest magnitude and the average survival percentage dropped from 100% in control to around 23%.

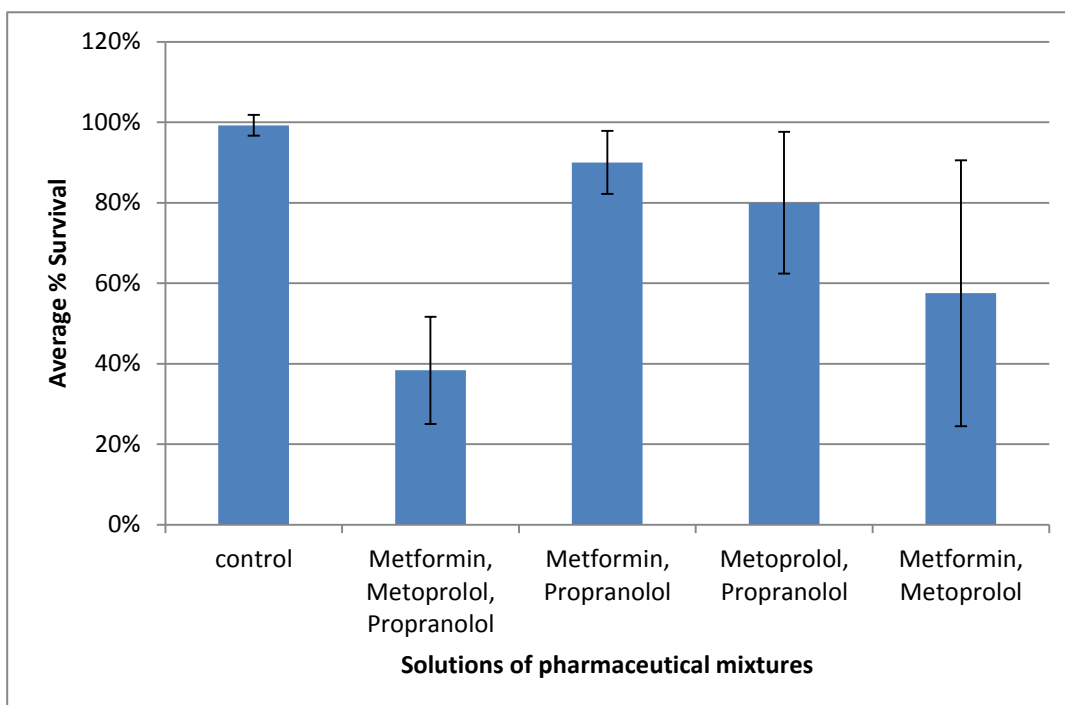


Figure 13: Synergistic effect of Metformin (50 ppm), Metoprolol (50 ppm) and Propranolol (1 ppm) mixtures on survival of 24 hour neonate *Daphnia magna* in a 48 hour exposure.

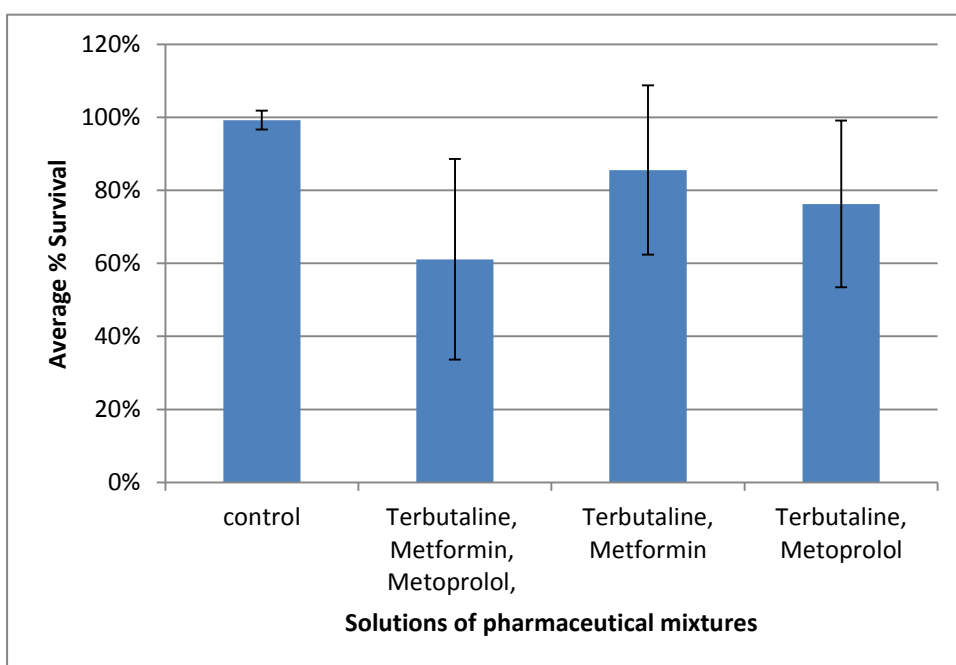


Figure 14: Synergistic effect of Terbutaline (100 ppm), Metformin (50 ppm) and Metoprolol (50 ppm) mixtures on survival of 24 hour neonate *Daphnia magna* in a 48 hour exposure.

In contrast to Figure 8, the low concentration complex mixture exposure (see Figure 13) suggests that the interaction of metformin and metoprolol at environmentally relevant concentrations is synergistic. This underscores an important point: the standard method for assessing mixture toxicity, using high concentrations of binary mixtures, does not accurately represent the ‘real’ environment. The interaction between chemicals in a mixture may be completely different at low, environmentally-relevant concentrations.

CONCLUSION

The differing metoprolol-metformin interactions shown in this study for high concentration and low concentration mixtures suggests that perhaps studying environmentally relevant exposures may be a more accurate representation of what happens in the aquatic environment. Future work will be aimed at testing additional drugs in binary and more complex mixtures to further elucidate this conclusion.

Toxicity endpoints other than mortality, including changes to growth and development, may occur at concentrations lower than LC_{50} values, or if chronic exposure occurs. In the experiments each drug was incubated at the NOAEL concentration: terbutaline (100 ppm), metformin (50 ppm), metoprolol (50 ppm) and propranolol (1 ppm). Synergism was observed in many of the mixtures, as shown above.

Additionally, further experiments need to be conducted with more complex mixture combinations using different concentrations and future studies should be directed towards elucidating the mechanisms of the observed toxicity and drug-drug interactions. Additional studies should also consider the toxicological impact of degradation products of drugs on aquatic life, the impact of drugs on other organisms and on the complete aquatic ecosystem. Future studies could also focus on utilizing better analytical techniques to reduce the experimental error and get more accurate and precise results. Furthermore, some pharmaceuticals target a specific biochemical site of action and the toxic effects observed may differ based on the sensitivity of the organism. Subsequently, pharmaceuticals may not be harmful to particular organisms; however, it may cause sub-lethal toxicity in organisms with genetic similarities. Thus,

future studies could assess synergism and antagonism in toxicity of mixtures of pharmaceuticals on other aquatic organisms.

Due to the persistence and increasing use of pharmaceuticals, they are increasingly more likely to be an environmental concern. Thus the research done in this field could form the foundation for better environmental regulation for pharmaceuticals in the wastewater and aquatic environment, particularly taking mixture effects into account.

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